REMARKS

Claims 1 – 20 are pending in the application. Claims 7 – 12 have been withdrawn from further consideration by the Examiner as being drawn to non-elected inventions. Claims 1 – 6 and 13 – 20 are under current examination. Claims 14 and 17 have been amended. Claims 21 and 22 have been added. No new matter has been added by virtue of the amendments and new claims, support being found throughout the specification and claims as originally filed. Support for new claims 21 and 22 can be found in the specification, for example at page 42, starting at line 29.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Objections

The abstract of the disclosure has been objected to because it does not commence on a sheet separate from other materials of the disclosure. Although it is unclear from the PAIR that the abstract is in not on a separate page, Applicants have made appropriate amendment to the specification to place the Abstract on a separate sheet of paper, and respectfully request that the objection be withdrawn.

The specification has been objected to for containing sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2) but are not present in the Sequence Listing and/or identified in the specification by sequence identifier numbers. Applicants submit herein a Sequence Listing in compliance with 37 CFR 1.821(a)(1) and (a)(2).

Claim Rejections- 35 U.S.C. § 112

Claims 14, 17 and 18 have been rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the enablement requirement. The Office Action argues that "the specification, while being enabling for treating prostate cancer in a

subject for intratumoral injection of a replication conditional adenovirus vector comprising a prostate-specific TRE operably linked to a nucleotide sequence encoding an E1A/AR chimeric protein, does not reasonably provide enablement for treating prostate cancer in a subject by administering said vector from a site remote from the tumor." (Office Action, p.5). Applicants respectfully disagree.

The instant claims are directed to a method of selectively lysing a neoplastic prostate cell, comprising contacting the cell with an effective amount of the adenoviral vector comprising a heterologous prostate-specific transcriptional regulatory element operably linked to a nucleotide sequence encoding an E1A/Androgen Receptor (AR) chimeric protein. The claims are directed to a method of producing a tissue-specific replication conditional adenovirus particle, the particle comprising a heterologous prostate-specific transcriptional regulatory element operably linked to a nucleotide sequence encoding an E1A/Androgen Receptor (AR) chimeric protein.

The specification clearly teaches the invention as claimed.

The adenovirus, as set forth in the claims, is a **tissue-specific** replication conditional adenovirus particle. Applicants direct the Examiner to paragraphs [0093] – [0094] of the specification, where Applicants clearly teach tissue specific replication dependent on the presence of a TRE whose activity is regulated by transcription factors in target tissue:

> The term "tissue-specific" is intended to mean that the transcriptional regulatory sequence to which the gene essential for replication is operably linked functions specifically in that tissue so that replication proceeds in that tissue. This can occur by the presence in that tissue, and not in non-target tissues, of positive transcription factors that activate the transcriptional regulatory sequence. It can also occur by the absence of transcription inhibiting factors that normally occur in non-target tissues and prevent transcription as a result of the transcription regulatory sequence. Thus, when transcription occurs, it proceeds into the gene essential for replication such that in that target tissue, replication of the vector and its attendant functions occur.

> As described herein, tissue specificity is particularly relevant in the treatment of the abnormal counterpart of a normal tissue. Such counterparts include, but are not limited to, cancerous prostate tissue and normal prostate tissue. Tissue specificity also includes the presence of an abnormal tissue type interspersed with normal tissue of a different tissue type, as for example in the case of metastases of prostate cancer, and the like, into tissue such as liver. In this case, the target tissue is the abnormal tissue, and tissue specificity reflects the restriction of vector replication to the abnormal tissue interspersed in the normal tissue. It is also to be understood that tissue specificity, in the context of treatment, is particularly relevant in vivo.

The specification clearly teaches one of skill in the art how to make an adenoviral particle comprising a heterologous prostate-specific TRE, as claimed. For example, at paragraph [0021] Applicants teach how to target the adenovirus to a specific site with a transcriptional regulatory element (TRE) and further, give examples of TREs that are useful in the invention. At [0137] – [0151] the specification describes TREs. Further, the specification described prostate specific TREs.

> In one embodiment, adenovirus vectors comprise heterologous TREs that are prostate cell specific. For example, TREs that function preferentially in prostate cells and can be used to target adenovirus replication to prostate neoplasia, include, but are not limited to, TREs derived from the prostate-specific antigen gene (PSA-TRE) (Henderson U.S. Pat. No. 5,698,443); the glandular kallikrein-I gene (from the human gene, hk2-TRE) (PCT US98/16312), and the probasin gene (PB-TRE) (PCT/US98/04132). All three of these genes are preferentially expressed in prostate cells and their expression is androgen-inducible. Generally, expression of genes responsive to androgen induction is mediated by an androgen receptor (AR).

The specification teaches at paragraph [0125] [0127] how to choose an adenoviral vector for use in the methods as claimed:

> The choice of adenoviral vector is primarily determined by the identity of the target cells, in this case prostate cells and therefore the type of cancer to be treated. As explained below in detail, an adenoviral vector comprising a PSA-TRE,

> PB-TRE, or hk2-TRE would preferentially replicate in prostate cells.

Moreover, the specification teaches, in particular, at paragraph [0130] that the adenoviral vectors that are chosen are those that preferentially replicate in TRE functional cells, and how to choose those vectors, and why this is useful in the context of cancer.

> The adenovirus vectors used in this invention replicate preferentially in TRE functional cells referred to herein as prostate cells. This replication preference is indicated by comparing the level of replication (i.e., titer) in prostate cells in which the TRE is active to the level of replication in cells in which the TRE is not active (i.e., a non-target cell). The replication preference is even more significant, as the adenovirus vectors used in the invention actually replicate at a significantly lower rate in TRE non-functional cells than wild type virus. Comparison of the adenovirus titer of a target cell to the titer of a TRE inactive cell type provides a key indication that the overall replication preference is enhanced due to the replication in target cells as well as depressed replication in non-target cells. This is especially useful in the cancer context, in which targeted cell killing is desirable. The TRE's preferably control genes necessary for replication, where the gene(s) necessary for replication is an early gene(s) of the adenovirus, preferentially the E1A gene.

The specification teaches what individuals are suitable for treatment and further, how to determine the presence of prostate cancer and the suitability of the individual for treatment by the methods described. For example, at paragraphs [0052] – [0053] the specification teaches that:

> Individuals suitable for treatment by these methods include individuals who have or are suspected of having prostate cancer, including individuals in the early or late stages of the disease, as well as individuals who have previously been treated or are about to undergo treatment (e.g., are in the adjuvant or neoadjuvant setting). Other individuals suitable for the methods described herein are those who are considered high risk for developing a prostate tumor, such as those who have a genetic predisposition to development of a neoplasia and/or who have been

> exposed to an agent(s) which is correlated with development of a neoplasia.
>
> The presence of prostate cancer and the suitability of the individual for receiving the methods described herein may be determined by any of the techniques known in the art, including diagnostic methods such as imaging techniques, analysis of serum tumor markers, and biopsy.

Next, having already described how to make an adenoviral particle comprising a heterologous prostate-specific TRE, how to choose an adenoviral vector for use in the methods as claimed, and, in particular how to choose those vectors that preferentially replicate in TRE functional cells, how to choose individuals that are suitable for treatment and to determine the presence of prostate cancer, the specification next discusses delivery of adenoviruses to the subject in need of treatment. Applicants point out in particular paragraphs [0171] – [0172] of the specification which describe how to deliver the adenoviral vectors to the target cell:

> Delivery of adenoviral vectors is discussed infra and is generally accomplished by either site-specific injection or intravenously. Direct intra-prostatic injections are preferred. Site-specific injections of either vector may include, for example, injections into the portal vein of the liver as well as intraperitoneal, intrapleural, intrathecal, intra-arterial, intra-tumor injections or topical application. These methods are easily accommodated in treatments using adenoviral vectors.
>
> The adenoviral vectors may be delivered to the target cell in a variety of ways, including, but not limited to, liposomes, general transfection methods that are well known in the art (such as calcium phosphate precipitation or electroporation), direct injection, and intravenous infusion. The means of delivery will depend in large part on the particular adenoviral vector (including its form) as well as the type and location of the target cells (i.e., whether the cells are to be transfected or transformed in vitro or in vivo).

Accordingly, the invention as claimed, and in particular the adenoviral vector comprising a heterologous **prostate-specific transcriptional regulatory element** operably linked to a nucleotide sequence encoding an E1A/Androgen Receptor (AR)

chimeric protein, methods of producing a tissue-specific replication conditional adenovirus particle, and methods of selectively lysing a neoplastic prostate cell, comprising contacting the cell with an effective amount of the adenoviral vector are supported by the specification.

Applicants respectfully request that the rejection be withdrawn.

Claim Rejections- 35 U.S.C. § 103(a)

Claims 1 – 6 and 13 – 20 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Rodriguez et al. (Cancer Res 1987; 57: 2559 – 63), in view of Suzuki et al. (Cancer Res 2001; 61: 1276 – 9) and Becker et al. (Mol Cell Bio 1989; 9:3878-87).

The claims are directed to a tissue-specific replication conditional adenovirus vector comprising a heterologous prostate-specific transcriptional regulatory element operably linked to a nucleotide sequence encoding an E1A/Androgen Receptor (AR) chimeric protein.

The Examiner argues that the Rodriguez reference teaches "a prostate-specific replication-competent adenoviral vector comprising a prostate-specific promoter driv(ing) the expression of E1A, and a method of using such to selectively supress prostate cancer cell growth." (Office Action, p.8). The Examiner admits that "Rodriguez does not teach fusing an E1A with an androgen receptor" but argues that "Suzuki supplemented the deficiency by arguing it was well known in the art that including a partial androgen receptor gene in a PSA gene construct enhances PSA promoter activity." (Office Action, p.9). Applicants respectfully disagree.

No combination of the cited references teaches the invention as claimed.

The instant invention is directed, in part, to a replication-competent target cell-specific adenoviral vector that comprises a chimera of an adenovirus gene essential for replication, the E1A gene, and the gene encoding the androgen receptor under transcriptional control of a target cell specific transcriptional regulatory element (TRE).

The invention is based particularly on E1A/Androgen receptor chimeras. As described in the specification, **E1A is known to both activate and inhibit trans-gene expression, by at least two different mechanisms** (see, e.g. paragraph [0116]). The specification clearly teaches that the knowledge of E1A in the art was unpredictable, given the variable reports of its activation or inhibition of trans gene expression. The specification sets forth Applicants hypothesis that "the reason we did not see androgen induction in the early prostate specific oncolytic vectors was because E1A was specifically inhibiting AR activity, a finding we later confirmed." (Paragraph [0116]).

Accordingly, Applicants direct the Examiner to the Examples section, beginning at paragraph [0192] of the published application, where **Applicants teach E1A as a co-activator of gene expression.** Example 2, at paragraph [0194], shows AR-E1A fusion chimera enhances AR dependent gene activation:

> **We tested our hypothesis that a fusion of E1A and AR domains would convert E1A into a potent co-activator** by performing co-transfection of our fusion constructs with an AR dependent reporter construct pBK-PSE-PBN-LUC (FIG. 3)... The full-length AR fusion (E1A-AR) demonstrated an excellent activation with hormone (super-induction), both in the AR positive cell line LNCaP and the AR negative cell line PC3. The E1A-DBD raised basal expression of the reporter slightly in all cell lines in a non-specific and hormone independent fashion. Importantly, the HeLa line failed to demonstrate much activity of any of the constructs with or without androgen. Subsequent experiments confirm the same pattern in LAPC4 as LNCaP and lack of activity in the colon cancer cell line DLD. Thus, the fusion of the E1A-AR is able to convert the AR negative cell line PC3 into an active inducer of prostate specific promoter activity, while maintaining specificity of action.

As taught above, it was **not obvious** at the time of the invention that E1A was a co-activator of gene expression, and it was **only obvious in view of the work presented in this application** that fusion of E1A with AR enhances AR dependent gene activation. Here, the Examiner's rejection is improperly based on a hindsight reconstruction of the invention in view of the applicant's own disclosure.

The Suzuki reference merely teaches expression of the partial androgen receptor (ARf) in a plasmid vector to transactivate the PSA gene without androgens. Suzuki provide no teaching or suggestion to express ARf with an adenoviral gene under the control of a TRE. In fact, Suzuki provide no teaching or suggestion to express Arf in any viral vector, and teach away from such use (p.1279).

The Becker reference does not make up for the flaws of the Rodriguez or Suzuki references to teach the invention as claimed. The Examiner argues that "Becker supplemented Rodriguez in view of Suzuki by establishing it was well known in the art that fusion of adenovirus E1A to a hormone receptor creates a hormonally inducible viral transactivator." (Office Action, p.9). The Examiner alleges that "it would have been reasonably suggested to the skilled person in the pertinent art to fuse the E1A with AR in the construct taught by Rodriguez to create an androgen hormonal inducible E1A expression construct ass taught by Becker, with a reasonable expectation of success" with motivation "to do so because androgen enhances PSA promoter activity as taught by Suzuki, and androgen increases the titer and specificity of the prostate replication conditional adenovirus as taught by Rodriguez." (Office Action, p.10).

The Becker reference is directed to fusion of E1A to **glucocorticoid receptor**. Nowhere does Becker teach that fusion of adenovirus E1A to a hormone receptor creates a hormonally inducible viral transactivator as claimed by the Examiner. Protein and peptide hormones, catecholamines and eicosanoids are all hormones that bind receptors. As not all hormone receptor are the same, glucocorticoid receptor and androgen receptor are not analagous. Applicants point out that the Becker reference is from 1989, and the knowledge of E1A has advanced significantly since this time. Accordingly, Applicants teach at paragraph [0116] of the specification that E1A is known to both activate and inhibit certain trans-gene expression:

> There appear to be at least two different mechanisms by which this can occur (Nat Rev Mol Cell Biol 2002; 3:441-52; Oncogene 2001; 20:7824-35): (1) sequestration of chromatin remodeling/HAT complexes required by certain transcription factors; (2) active inhibition of certain trans-genes by direct binding of E1A with the transcription factor and the inhibitory

> complex C-terminal binding protein (CtBP). CBP/p300 and PCAF are not required for expression of all genes, but rather only a small subset of genes regulated by transcription factors thought to be most important in differentiation. Two-prostate specific oncolytic adenoviruses, CN706 (also known as CG7060) and CV787 (also known as CG7870) are unable to undergo androgen induction for enhanced oncolytic activity (Cancer Res 1999; 59:4200-3). This finding is surprising given that they are regulated by ARE containing promoters. In contrast, generation of an estrogen receptor dependent conditionally replication competent adenovirus, by placing a portion of the pS2 promoter upstream of the E1A gene, resulted in a viral construct whose oncolytic activity was markedly enhanced by estrogen and antagonized by anti-estrogen (Hum Gene Ther 2000; 2009-24)...Simply stated, E1A is capable of either up-regulating or down-regulating retinoic acid responsive target genes dependent on the particular target gene promoter and the nature of the retinoic acid receptor complex (RXR versus RAR).

Accordingly, no combination of the cited references teaches the invention as claimed.

Early consideration and allowance of the application are earnestly solicited.

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Respectfully submitted,

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